CLAIMS

What is claimed is:

1. A modified enzyme wherein one or more amino acid residues in the enzyme are replaced by cysteine residues, wherein the cysteine residues are modified by replacing the thiol hydrogen in said cysteine residues with a substituent group providing a thiol side chain comprising a multiply charged moiety.

2. The enzyme of claim 1, wherein the enzyme is a serine hydrolase.

3. The enzyme of claim 2, wherein the enzyme is a protease.

4. The enzyme of claim 3, wherein the protease is a subtilisin.

5. The enzyme of claim 4, wherein said subtilisin is a *Bacillus lentus* subtilisin.

6. The enzyme of claim 1, wherein the amino acid replaced with a cysteine is an amino acid selected from the group consisting of asparagine, leucine, methionine, and serine.

7. The enzyme of claim 1, wherein the amino acid replaced with a cysteine is in a binding site of the enzyme.

8. The enzyme of claim 7, wherein the amino acid is in a subsite selected from the group consisting of S_1, S_1', and S2.

9. The enzyme of claim 1, wherein said enzyme is a subtilisin-type serine hydrolase and said cysteine is substituted for the amino acid corresponding to a *Bacillus lentus* subtilisin residue selected from the group consisting of residue 156, reside 166, residue 217, residue 222, residue 62, residue 96, residue 104, residue 107, reside 189, and residue 209.

10. The enzyme of claim 1, wherein said enzyme is a trypsin-chymotrypsin-type serine protease and said cysteine is substituted for the amino acid

corresponding to a trypsin residue selected from the group consisting of Tyr94, Leu99, Gln175, Asp189, Ser190, and Gln192.

11. The enzyme of claim 1, wherein said enzyme is an alpha/beta serine hydrolase and said cysteine is substituted for the amino acid corresponding to a *Candida antartica* lipase (Protein Data Bank entry 1tca) residue selected from the group consisting of Trp104, Thr138, Leu144, Val154, Ile189, Ala 225, Leu278 and Ile185.

12. The enzyme of claim 1, wherein the multiply charged moiety is negatively charged.

13. The enzyme of claim 12, wherein the multiply charged moiety is selected from the group consisting of sulfonatoethyl thiol, 4-carboxybutyl thiol, 3,5-dicarboxybenzyl thiol, 3,3-dicarboxybutyl thiol, and 3,3,4-tricarboxybutyl thiol.

14. The enzyme of claim 12, wherein the multiply charged moiety is a dendrimer or a polymer.

15. The enzyme of claim 1, wherein the multiply charged moiety is positively charged.

16. The enzyme of claim 15, wherein the multiplyh charged moiety is selected from the group conisisting of aminoethyl thiol, 2-(trimethylammonium)ethyl thiol, 4,4-bis(aminomethyl)-3-oxo-hexyl thiol, and 2,2-bis(aminomethyl)-3-aminopropyl thiol.

17. The enzyme of claim 15, wherein the multiply charged moiety is a dendrimer or a polymer.

18. A method of producing a modified enzyme, said method comprising:

providing an enzyme wherein one or more amino acids have been replaced with cysteine residues; and

replacing the thiol hydrogen, in said one or more cysteine residues, with a substituent group providing a thiol side chain comprising a multiply charged moiety.

19. The method of claim 18, wherein the enzyme is a serine hydrolase.

20. The method of claim 19, wherein the enzyme is a protease.

21. The method of claim 20, wherein the protease is a subtilisin.

22. The method of claim 21, wherein said subtilisin is a *Bacillus lentus* subtilisin.

23. The method of claim 18, wherein the amino acid replaced with a cysteine is an amino acid selected from the group consisting of asparagine, leucine, methionine, and serine.

24. The method of claim 18, wherein the amino acid replaced with a cysteine is in a binding site of the enzyme.

25. The method of claim 24, wherein the amino acid is in a subsite selected from the group consisting of S_1, S_1', and S2.

26. The method of claim 18, wherein said enzyme is a subtilisin-type serine hydrolase and said cysteine is substituted for the amino acid corresponding to a *Bacillus lentus* subtilisin residue selected from the group consisting of residue 156, reside 166, residue 217, residue 222, residue 62, residue 96, residue 104, residue 107, reside 189, and residue 209.

27. The method of claim 18, wherein said enzyme is a trypsin-chymotrypsin-type serine protease and said cysteine is substituted for the amino acid corresponding to a trypsin residue selected from the group consisting of Tyr94, Leu99, Gln175, Asp189, Ser190, and Gln192.

28. The method of claim 18, wherein said enzyme is an alpha/beta serine hydrolase and said cysteine is substituted for the amino acid corresponding to a *Candida antartica* lipase (Protein Data Bank entry 1tca) residue selected from the group consisting of Trp104, Thr138, Leu144, Val154, Ile189, Ala 225, Leu278 and Ile185.

29. The method of claim 18, wherein the multiply charged moiety is negatively charged.

30. The method of claim 29, wherein the multiply charged moiety is selected from the group consisting of sulfonatoethyl thiol, 4-carboxybutyl thiol, 3,5-dicarboxybenzyl thiol, 3,3-dicarboxybutyl thiol, and 3,3,4-tricarboxybutyl thiol.

31. The method of claim 29, wherein the multiply charged moiety is a dendrimer or a polymer.

32. The method of claim 18, wherein the multiply charged moiety is positively charged.

33. The method of claim 32, wherein the multiply charged moiety is selected from the group consisting of aminoethyl thiol, 2-(trimethylammonium)ethyl thiol, 4,4-bis(aminomethyl)-3-oxo-hexyl thiol, and 2,2-bis(aminomethyl)-3-aminopropyl thiol.

34. The method of claim 32, wherein the multiply charged moiety is a dendrimer or a polymer.

35. A composition comprising the enzyme of any one or claims 1 through 17 and a detergent.

36. A method of assaying for a preferred enzyme, said method comprising:

a) providing a swatch of material comprising a piece of material and a stain;

b) fixing the stain to the material;

c) applying an enzyme to the swatch; and

d) incubating the watch and the enzyme.

37. The method of claim 36, further comprising determining the degree of removal of the stain from the material.

38. The method of claim 36, wherein the enzyme is selected from the group consisting of a protease, a cellulase, an amylase, a laccase, and a lipase.

39. The method of claim 36, wherein the enzyme is a modified hydrolase wherein one or more amino acid residues in the hydrolase are replaced by cysteine residues,

wherein the cysteine residues are modified by replacing the thiol hydrogen in said cysteine residues with a substituent group providing a thiol side chain comprising a multiply charged moiety.

40. The method of claim 36, wherein the material is selected from the group consisting of a fabric, plastic, or ceramic.

41. The method of claim 36, wherein the stain comprises a component selected from the group consisting of blood, milk, ink, grass, gravy, chocolate, egg, cheese, clay, pigment, and oil.

42. The method of claim 41, wherein the stain is a blood/milk/ink (BMI) stain.

43. The method of claim 36, wherein said fixing comprises incubating said stain with a cross-linking agent.

44. The method of claim 36, wherein the enzyme is applied to the swatch in combination with a detergent ingredient.

45. The method of claim 36, further comprising agitating the swatch and enzyme during incubation.

46. A method of assaying for a preferred detergent composition, said method comprising:

a) providing a swatch of material comprising a piece of material and a stain;

b) fixing the stain to the material;

c) applying a detergent composition to the swatch; and

d) incubating the watch and the detergent composition.

47. The method of claim 46, further comprising the determining the degree of removal of the stain from the material.

48. The method of claim 46, wherein the material is selected from the group consisting of a fabric, plastic, or ceramic.

49. The method of claim 46, wherein the stain comprises a component selected from the group consisting of blood, milk, ink, grass, gravy, chocolate, egg, cheese, clay, pigment, and oil.

50. The method of claim 49, wherein the stain is a blood/milk/ink (BMI) stain.

51. The method of claim 46, wherein said fixing comprises incubating said stain with a cross-linking agent.

52. The method of claim 46, wherein the enzyme is applied to the swatch in combination with an enzyme.

53. The method of claim 52, wherein the enzyme is selected from the group consisting of a protease, a cellulase, an amylase, a laccase, and a lipase.

54. The method of claim 52, wherein the enzyme is a modified hydrolase wherein one or more amino acid residues in the hydrolase are replaced by cysteine residues, wherein the cysteine residues are modified by replacing the thiol hydrogen in said cysteine residues with a substituent group providing a thiol side chain comprising a multiply charged moiety.

55. The method of claim 46, further comprising agitating the swatch and detergent composition during incubation.

56. A method of determining the catalytic efficiency of an enzyme, said method comprising:

a) providing a swatch of material comprising a piece of material and a stain;

b) applying the enzyme to the swatch;

c) incubating the swatch and the enzyme;

d) removing the swatch or supernatant; and

e) measuring a constituent of the stain.

57. The method of claim 56, wherein the enzyme is selected from the group consisting of a protease, a cellulase, an amylase, a laccase, and a lipase.

58. The method of claim 56, wherein the enzyme is a modified hydrolase wherein one or more amino acid residues in the hydrolase are replaced by cysteine residues, wherein the cysteine residues are modified by replacing the thiol hydrogen in said cysteine residues with a substituent group providing a thiol side chain comprising a multiply charged moiety.fixing the stain to the material.

59. The method of claim 56, wherein the material is selected from the group consisting of a fabric, plastic, or ceramic.

60. The method of claim 56, wherein the stain comprises a component selected from the group consisting of blood, milk, ink, grass, gravy, chocolate, egg, cheese, clay, pigment, and oil.

61. The method of claim 41, wherein the stain is a blood/milk/ink (BMI) stain.

62. The method of claim 56, wherein the stain is applied to the swatch in combination with a detergent ingredient.

63. The method of claim 56, wherein the constituent is ink from a BMI stain.

64. The method of claim 56, wherein the constituent is labeled blood from a BMI stain.

65. The method of claim 56, wherein the constituent is in the supernatant.

66. The method of claim 56, wherein the constituent is measured by absorbance of the constituent.

67. The method of claim 56, wherein the constituent is measured by fluorescence of the constituent.

68. The method of claim 56, wherein the stain is fixed to the fabric by incubation with a cross-linking agent.

69. A kit comprising a container containing a modified enzyme wherein one or more amino acid residues in the enzyme are replaced by cysteine residues, wherein the cysteine residues are modified by replacing the thiol hydrogen in said cysteine residues with a substituent group providing a thiol side chain comprising a multiply charged moiety.

70. A kit comprising a container containing a methane sulfonate reagent comprising a multiply charged substituent and instructional materials teaching the use of the sulfonate reagent to couple a mutiply charged moiety to a cysteine residue in a protein.